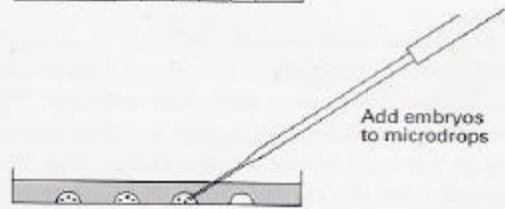
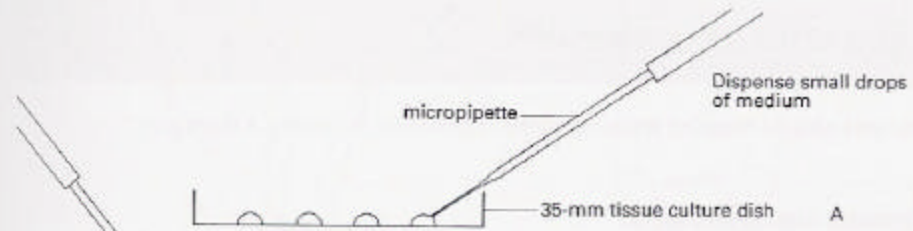
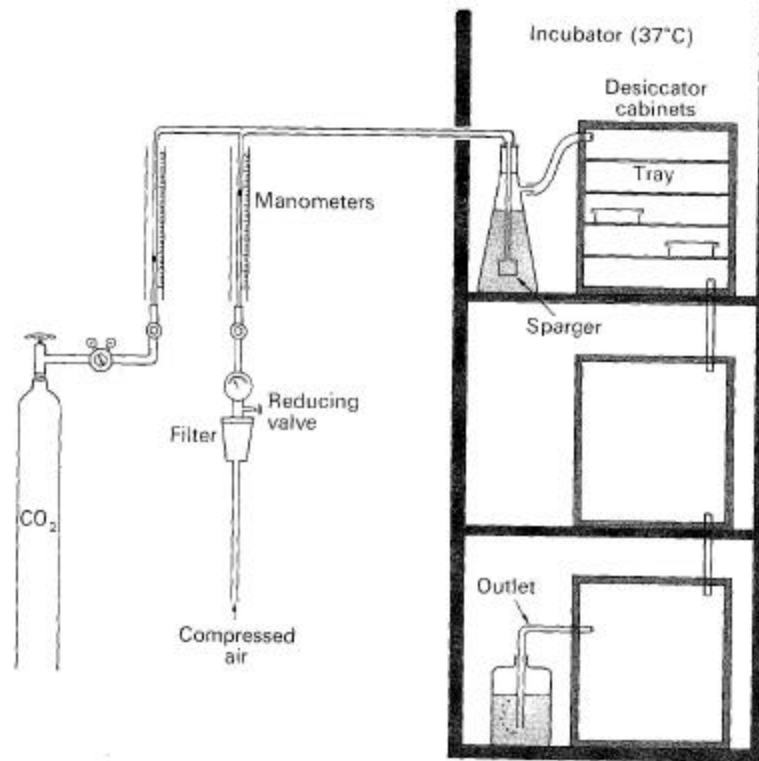


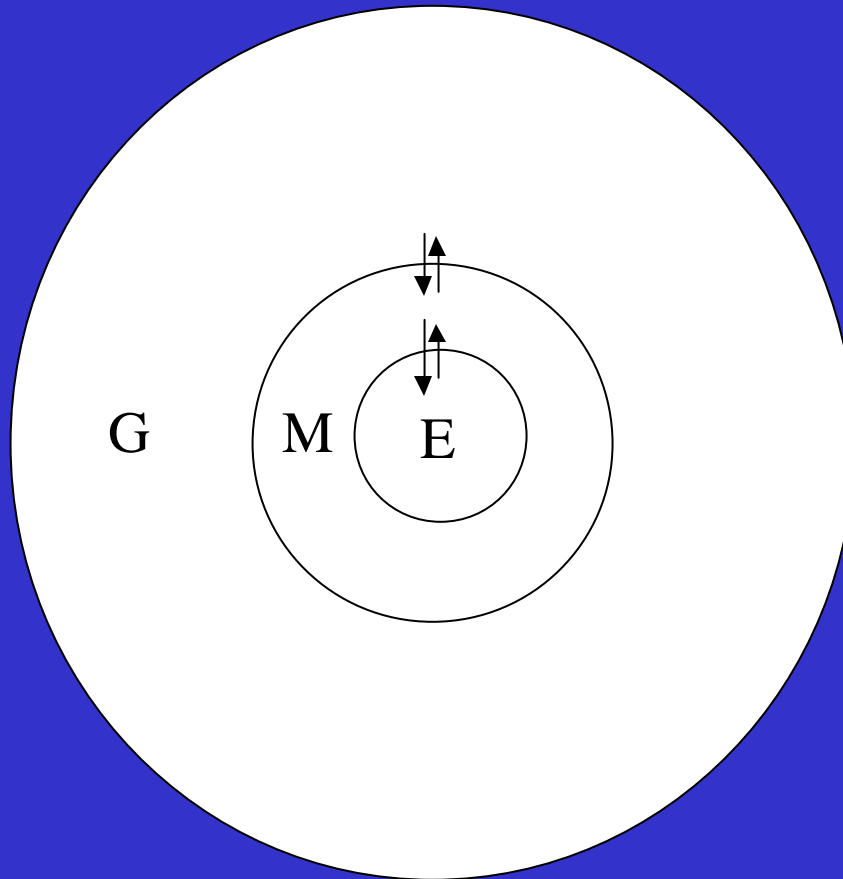
Culture of Preimplantation Embryos

John D. Biggers





Theoretical Model



Definitions

- Biological media
 - Chemically defined media
 - Protein-free media
-
- Simple ≤ 12
 - Complex >12

Reasons for Using a Chemically Defined Medium

(Modified from Lewis and Lewis ,1911)

- Can be easily reproduced at different times and in different laboratories.
- Can be varied in a controlled manner by selecting compounds and their concentrations.
- Are free of unknown enzyme activities, *and hormones and growth factors*, which may interfere with the responses being studied.

Quotation from Edwards et al. (1980)

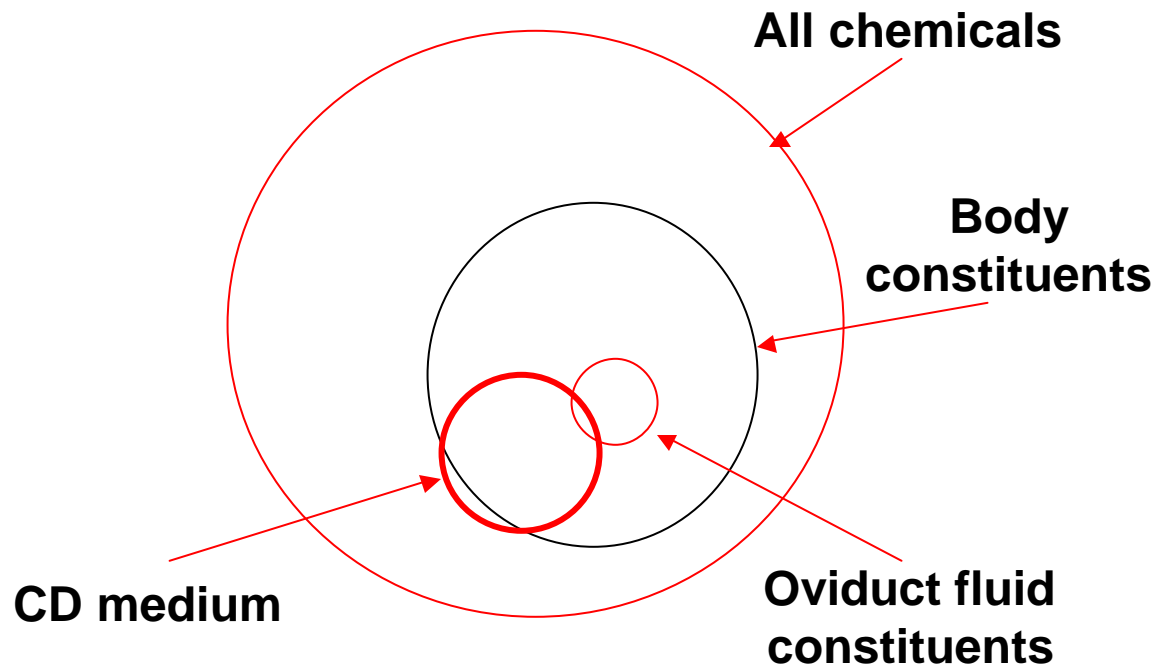
- “...human ova will tolerate a wide range of culture media. The most suitable and simple medium for fertilization is Earle’s medium supplemented with pyruvate and inactivated human serum. The same medium can be used for cleavage, with higher concentrations of serum.....Ham’s F10 is unnecessary...”

Specifically designed chemically defined culture media

- Complex medium:
Medium B2/B3 (Menezo, Testart and Perrone, 1984)
- Simple medium:
- HTF (Quinn, 1985)

Later Methods for the Culture of Human Preimplantation Embryos

- Introduction of co-culture on Vera cells (Menezo, Guerin and Czyba, 1990).
- Introduction of media containing no glucose and phosphate (Quinn, 1995).
- Introduction of a 2-step culture method (Gardner and Lane, 1997).



**The Classes of Components of a Chemically
Defined Medium for the Culture of
Preimplantation Embryos**

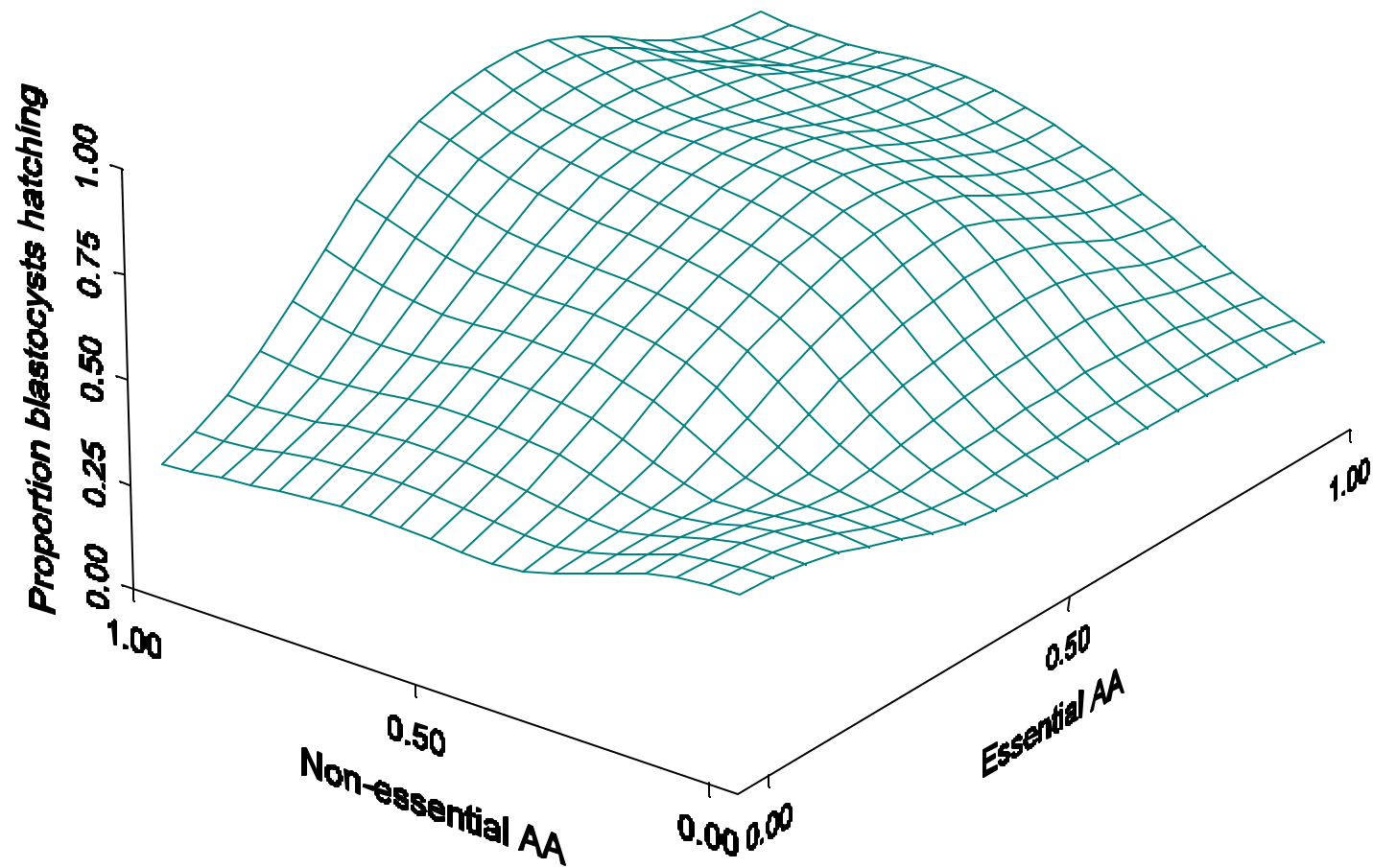
Medium KSOM – a Simple Medium

Component	Concentration (mmol/l)
NaCl	95
KCl	2.5
KH ₂ PO ₄	0.35
MgSO ₄	0.2
CaCl ₂	1.71
NaHCO ₃	25
Lactate	10
Pyruvate	0.2
Glucose	0.2
Glutamine	1
EDTA	0.01
BSA	1 mg ml ⁻¹

Strategies for the design of media

- Let the embryos choose strategy
- Back to nature strategy

Concentration-response Surface



It is inevitable that the embryos are stressed

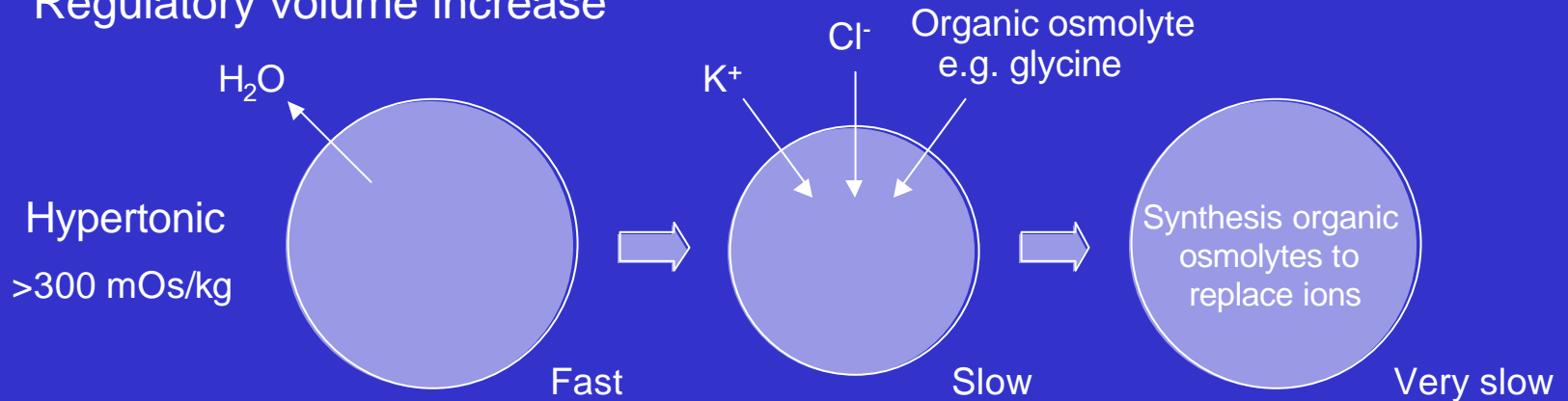
- It is very unlikely that we can completely recreate the chemical environment to which the embryos are exposed as they travel from the site of fertilization to the uterus
- All we can do is optimize the composition of chemically defined media to minimize the stress

Example of a toxic effect

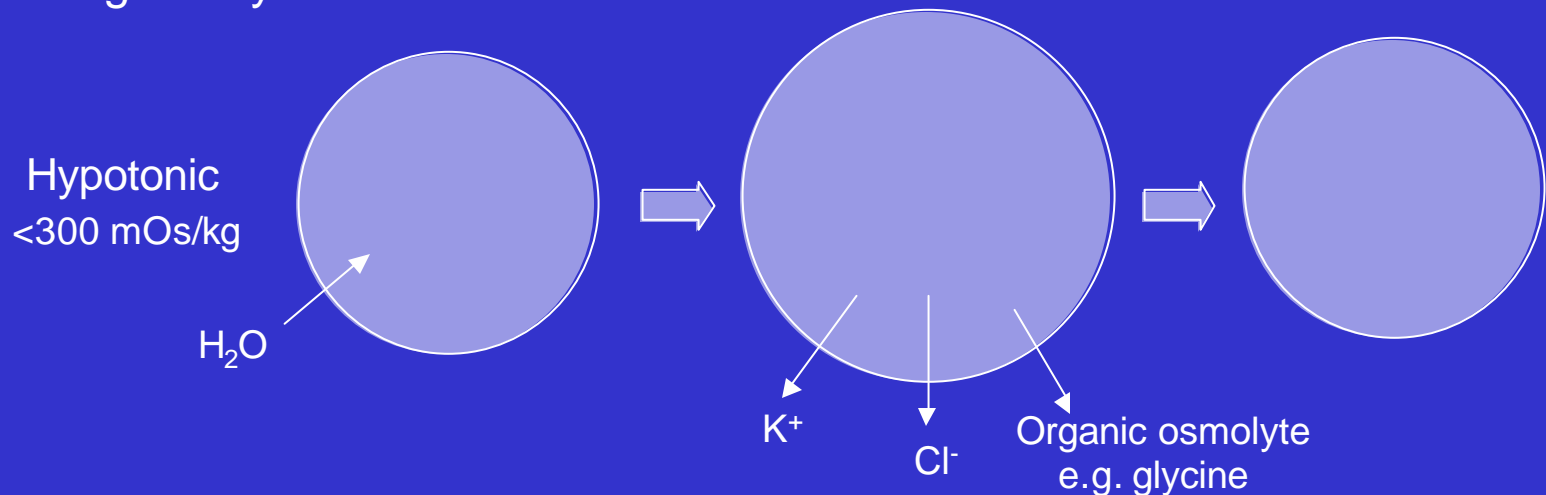
- Glutamine has been reported to increase the incidence of exencephaly (Gardner and Lane, 1995).
- Replacement with a dipeptide containing glutamine, e.g. glycylglutamine (GlyGln).

Mild Stress - Cell Volume Regulation

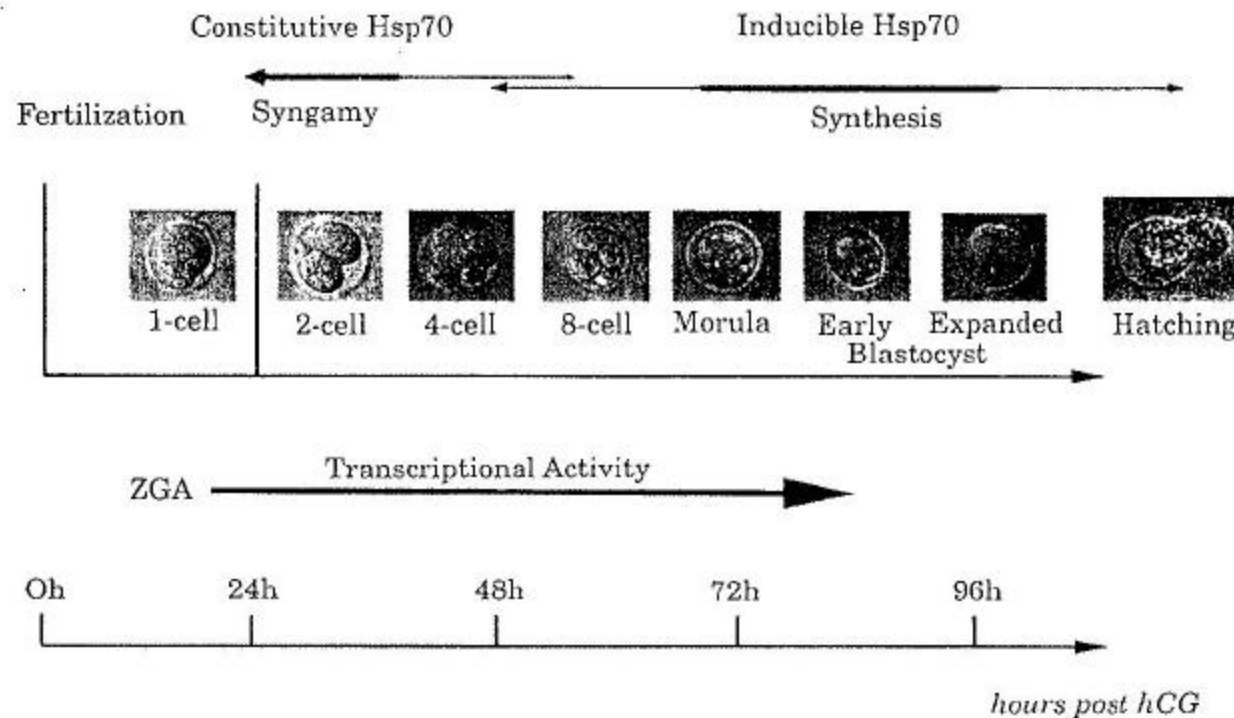
Regulatory volume increase



Regulatory volume decrease



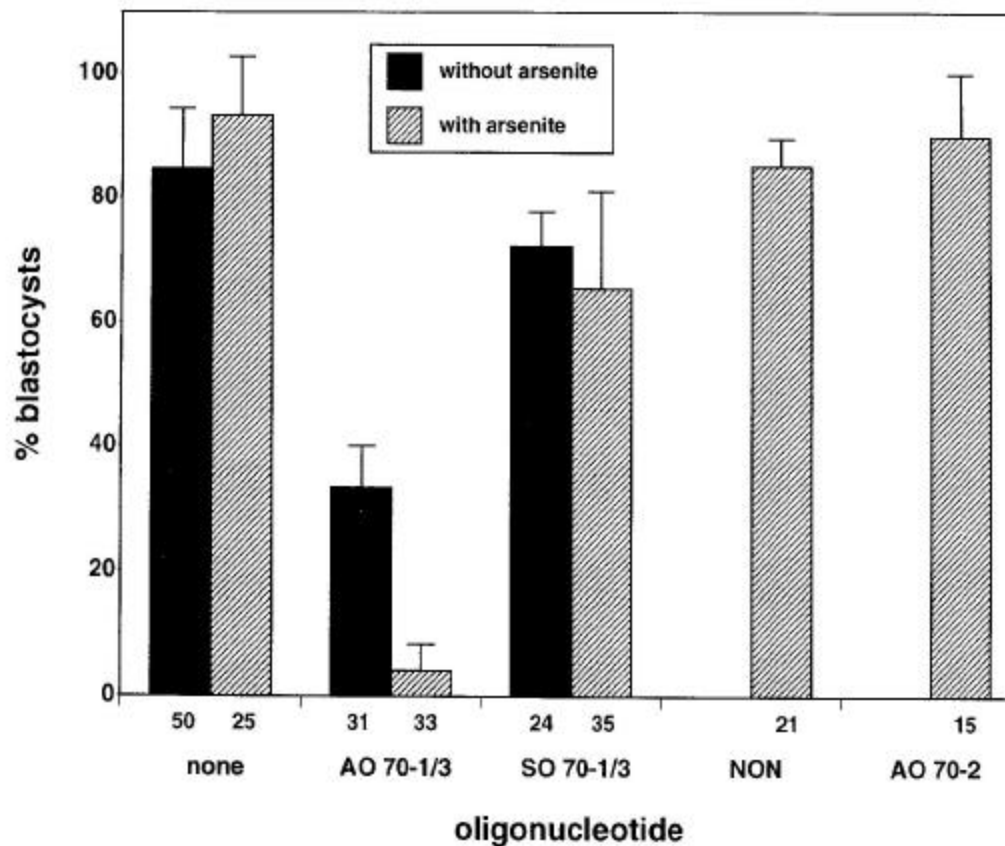
Hsp70 Expression in the Preimplantation Mouse Embryo



From Luft and Dix (1999)

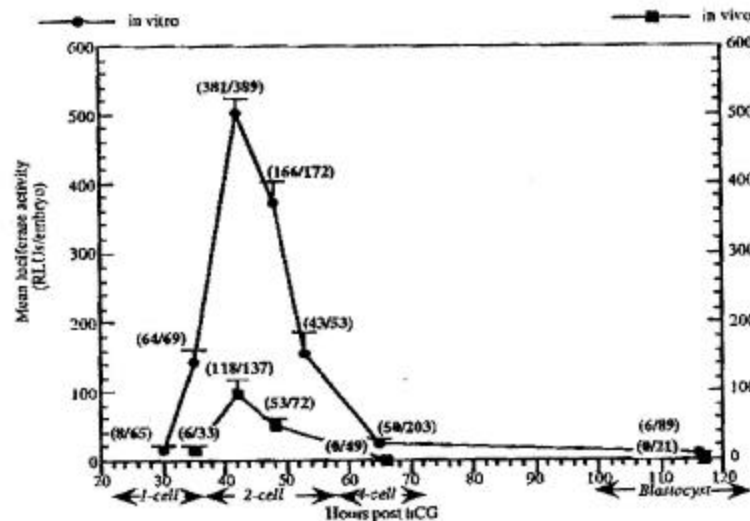
Effect of Antisense Hsp70 on Blastocysts Produced from Zygotes in KSOM

(From Dix et al., 1998)



Effect of medium M16 on HSP 70.1

From Christians et al., 1995



Genetic Examples

- Differential effects of media on gene action
Whitten's medium v. KSOM^{AA}
- Imprinting genes

H19 gene Expression in Preimplantation Embryos Cultured in Whitten's Medium and KSOM^{AA}

- H19 is preferentially expressed from the maternal allele (imprinting).
- H19 is abnormally expressed from both the paternal and maternal alleles using Whitten's medium.
- H19 is normally expressed only by the maternal allele when KSOM^{AA} is used.

Factors affecting responses

- The substances in the medium
- Their concentrations



An Ethical Issue

- Secrecy about the composition of media.